MOLDINESS IN BUTTER

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INTRODUCTION

References to mold in butter are not uncommon in dairy literature, but specific information is lacking as to what forms of mold occur on butter, the conditions which permit mold development, and the actual changes produced in the butter. As met in the market, losses from mold take two forms: (1) The growth of mold upon the tub, lining, or wrapper injures the appearance and salability of the package without seriously affecting the quality of its contents. (2) Mold development upon the butter itself when continued for a considerable period produces changes which can not be eliminated even by the renovation process. Such butter becomes an actual loss. The work reported here aims to cover the biological phases of this problem. The study of the chemical changes produced in the butter will be reported later.

ORIGIN OF BUTTER SAMPLES

Characteristic samples representing the range of conditions and appearances found in commercial butter were obtained through the inspection service of the Dairy Division. These were examined in the mycological laboratory. The number and variety of mold colonies upon each sample were noted and cultures were made to obtain the species represented. The samples were then taken to the chemical laboratory for analysis. Consideration of the known factors influencing mold growth called for the determination of the quantities of water and protein available, together with the percentage of salt as a possible limiting factor.

In Table I are given the analyses of samples of moldy butter from several sources.

TABLE I.—*Analyses of samples of moldy butter*

Sample No.	Water.	Salt.	Curd.[a]	Sample No.	Water.	Salt.	Curd.[a]
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
3515.....	11. 65	1. 98	1. 48	3554s.....	7. 38	0. 63	0. 57
3529.....	12. 00	. 65	1. 53	3546a.....	16. 02	3. 05	. 68
3530.....	9. 40	2. 19	. 64	3546b.....	18. 00	1. 40	. 75
3554g.....	11. 09	2. 66	. 66	3546c.....	16. 02	3. 50	. 62
3554r.....	11. 72	2. 13	. 64				

[a] The term "curd," as used in this paper, means the amount of nitrogen multiplied by the factor 6.38.

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Samples Nos. 3546a, 3546b, and 3546c were taken from a tub of butter containing three small churnings. The tub had been kept in a refrigerator for three or four months and was very rancid. Since the butter was designed for packing stock to be used in experimental work on renovated butter, no particular care was taken in its manufacture. It happened that the top layer (3546a) and bottom layer (3546c) were heavily salted, while the middle layer (3546b) contained but a small percentage of salt and a considerably higher percentage of water. The top and bottom layers were free from mold; the middle layer showed areas typically representing each of the types of moldiness described in the following pages.

TYPES OF MOLD FOUND IN SAMPLES

From the study of these and other available samples of moldy butter three well-marked types of mold effects are distinguished:

1. SMUDGED, OR ALTERNARIA, TYPE.—In samples Nos. 3515, 3546b, and 3554s, dark, smoky, or rarely greenish colors occurred in patches which suggested soot or dirty-finger marks. Microscopic examination showed mold mycelium with dark-brown or green walls on or under the surface. Frequently these colonies are entirely submerged in the butter. Sometimes hyphæ were observed 4 to 5 mm. below the surface. Spores were rarely found, but colonies transferred to culture media grew freely and fruited normally. The dark-brown or black hyphæ were the most common and proved to be species of Alternaria. Where a greenish color was seen species of Cladosporium developed. These submerged areas suggest the appearances noted by Patterson (1900),[1] and attributed to *Stemphylium butyri* Patterson. The occurrence of Cladosporium in butter has been studied by Jensen (1900) and the organism found was named by him "*Cladosporium butyri.*" The species of Cladosporium, however, are abundant upon all kinds of roughage fed to cows, and the spores find entrance to the milk from the handling of such feed by the milkers. One of the writers had access to cultures made from many samples of cream by the bacteriologists of the Storrs Agricultural Experiment Station some years ago. In these cultures colonies of Cladosporium were so abundant as to indicate that spores of these species remain with the cream after separation. Species of Alternaria are very common in the same circumstances and appeared in these cultures, but less abundantly. Their appearance as colonies in the butter, therefore, is due to the ability of these species to grow in the very severe conditions imposed by a mass of butter. One of the writers has found a species of Alternaria growing and fruiting in a box of shoe paste. Species of this group have also been isolated from various forms of fat when small inclusions of water occur. Few other graminicolous fungi seem able to produce colonies under these conditions, though spores of many kinds are undoubtedly present.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 310.

In at least one sample, contributed by Dr. G. P. Clinton, of the Connecticut Agricultural Experiment Station, and again in a sample of fat studied by Dr. C. N. McBryde, of the Bureau of Animal Industry, a fungus producing abundant orange to red mycelium and red blotches upon the butter was obtained. In butter and in the culture media used no spores have thus far been obtained. This organism grows under the same conditions as Alternaria.

2. GREEN-MOLD TYPE.—Green molds were found more or less frequently upon all the samples tabulated except Nos. 3546a and 3546c. Cultures of these molds proved to be species of Penicillium. Three common species were often found. These were *P. roqueforti*, a variety or strain of *P. expansum*, and *P. chrysogenum*. Several other forms difficult to identify were occasionally obtained. Aside from *P. roqueforti*, these are identifiable only by careful culture and comparison. These molds form green patches on the surface and follow seams or cracks into the mass. In one tub (No. 3515), where extensive moldy areas were found in cracks and seams, the presence of *P. roqueforti* was suggested by a strong odor and flavor resembling that of Roquefort cheese. Marked physical changes in the fat itself were noticeable. Culture confirmed the identification of the organism. So far as observed, no such extensive changes were produced by the other species. The storage of butter in tubs is accompanied by low percentages of free oxygen[1] in the butter suggestive of the conditions in Roquefort cheese (Thom and Currie, 1913). Mold is found upon the liners, upon the inside of the tub itself, and in the cracks of the butter. In all these places interchange of gases is very slow, thus favoring the dominance of Roquefort mold, which is more tolerant of such conditions than other species.

3. OIDIUM TYPE.—The third form produces various shades of orange-yellow discoloration, with little or no surface growth. Culture and microscopic examination show that these areas are produced by *Oidium lactis*. This organism grows to the depth of several millimeters within the mass of butter as a complex mycelium with hyphæ varying in diameter with the size of the spaces between the masses of fat. Some spores are formed and at times surface-fruiting areas. Bacterial activity is commonly associated with the presence of this mold.

BLACK MOLDS, OR MUCORS.—Where butter has been moist enough for loose masses of surface mycelium to grow, mucors are sometimes seen. These molds are found by culture to be present in many other samples in which no visible colonies are produced.

EXPERIMENTAL WORK

To study the conditions favorable to mold growth in butter special samples of butter were prepared, some low in water content and some high in water content, some thoroughly washed to reduce the curd con-

[1] Unpublished results of Dr. D. C. Dyer, of the Dairy Division.

tent and some with casein added to raise the protein content. One ounce of salt to 1 pound of butter was used in some samples; no salt in others. Slices of butter of each kind were put into Petri dishes and inoculated with a series of molds obtained from butter. Among these were *Oidium lactis*, *Mucor* sp., *Alternaria* sp., and several species of Penicillium. The dishes were then allowed to stand in an incubator at the temperature and relative humidity of the laboratory for several days. Absolutely no surface growth of mold was obtained. Part of these Petri dishes were then placed in moist chambers and it was found that mold colonies developed upon every sample so placed. These growths included not only the species inoculated into the butter but other forms whose spores were present in the butter as made. At the low relative humidities prevailing in the laboratories of the Dairy Division from February to April, 1914, no mold colonies were able to develop in butter representing a range in water content greater than the usual range of percentage in market butter.

The addition of 2 to 3 per cent of water to butter containing but 14 or 15 per cent does not make the quantity of water present sufficient to support mold growth aside from conditions of high humidity.

RELATION OF HUMIDITY TO MOLD GROWTH

In moist-chamber culture comparison between samples containing normal and low protein with samples containing excess or added protein showed that mold growth was more rapid and extensive when protein was added. The failure of molds to grow in these same cultures under the ordinary humidity conditions of the laboratory proved that the essential factor in molding was not protein, but water.

To define these humidity relations more closely, three desiccators were prepared in which definite relative humidities could be maintained. For this purpose the bases of the desiccators were filled with sulphuric acid standardized to the specific gravities—from Hastings's (1909) table—required to maintain, respectively, 90 per cent, 79.6 per cent, and 69.6 per cent relative humidity. Three samples were used: One sample of butter was made with low-salt content (0.55 per cent); one at normal salting (2.43 per cent); and one sample of butter fat, free from water, with skim-milk powder added.

The composition of these three samples is given in Table II.

TABLE II.—*Composition of samples of butter used in mold-growth tests*

Character of sample.	Water.	Salt.	Curd.
	Per cent.	*Per cent.*	*Per cent.*
Normal-salt butter	15.2	2.43	0.62
Low-salt butter	15.6	.55	.62
Butter fat + dry skim milk	None.	None.	.48

Three slices, one from each of these samples, were put into each one of a series of 24 Petri dishes. The three slices in each dish were thus under absolutely the same conditions. Six species of mold were then selected and were heavily inoculated into the plated slices—each slice in four Petri dishes being inoculated with one species of mold. Four sets of six dishes each were thus available. One set of six Petri dishes was put into a moist chamber (approximately 100 per cent of relative humidity), and one each into the desiccators at 90, 79.6, and 69.6 per cent relative humidity. The cultural results are given in Table III.

TABLE III.—*Effects of salt and humidity on mold growth in butter*[a]

Mold.	Growth in moist chamber.			Growth under relative humidities of—								
				90.6 per cent.			79.6 per cent.			69.6 per cent.		
	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.
	0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.	
Alternaria sp	0.9	0	0	0.3	0	0	0.6	0	0	0	0	0
Mucor sp	(?)	0	0	0	0	0	0	0	0	0	0	0
Oidium sp	[b]0.6		0	.5	0	0	.5	0	0	.3	0	0
Penicillium roqueforti	.5	0	0	.5	.2	0	0(?)	0	0	0(?)	0	0
Penicillium chrysogenum	.5	.1	.8	.4	.2	.3	.3	0	.2	.3	0	0
Penicillium expansum	.5	.1(?)	0	.3	0	0	.3	0	0	0	0	0

[a] A typical colony would be designated as 1.0; lesser growths by decimal fractions.
[b] Submerged.

Examination of this table shows that a single species, *Penicillium chrysogenum*, was able to produce a colony upon the butter fat plus the water obtainable from the air. Careful examination of the other samples showed no mold. In the low-salted butter, however, with 15.6 per cent of water marked growth occurs; the water in this butter is therefore to be regarded as an essential factor in the molding found here. The butter containing 2.43 per cent of salt shows determinable growth from but two species, *P. roqueforti* and *P. chrysogenum*. No growth of species of Alternaria, Oidium, or Mucor was found upon this butter. The low-salted butter shows very appreciable mold colonies of all species except the Mucor. Growth was greatest in the moist chamber. Nearly as good growth was obtained, however, with a relative humidity of 90.6 per cent, and considerable growth in four of the species with 79.6 per cent. In the presence of 69.6 per cent there was very little visible mold, even in the low-salted sample. The individual samples of low-salted butter all showed the characteristic orange-yellow colors due to the development of spores of *Oidium lactis*, which were evidently present from the first in all slices. In this experiment the organism grew only in its submerged form; hence, it was little affected by the relative humidity to which the other species responded so clearly. Alternaria and Oidium developed

only in the low-salted samples. Alternaria appeared in several places without inoculation. *Rhizopus nigricans* was found once in a moist-chamber sample. *Aspergillus fumigatus* and *A. niger* both appeared in one or more cases, but none of these species appeared where the percentage of salt was 2.43.

The same fact is illustrated on a larger scale by the tub of butter analyzed as No. 3546 in Table I. Of the three samples packed together in one tub the middle layer was low-salted and typically moldy, while the top and bottom layers were free from mold.

These results harmonize fully with the data from the analysis of butter as found in Table I and with the preliminary cultural data as given in subsequent experiments. Two of the three types of moldiness, the smudged and the orange-yellow forms, occur only in butter containing less than 2 per cent of salt. Even with green molds under high humidities and at temperatures far above those used in storage, growth in these experiments was negligible in butter with a salt content of 2.43 per cent.

THE SALT FACTOR IN MOLD GROWTH

The salt factor in butter is calculated as follows: Thompson, Shaw, and Norton (1912), in analyzing 695 samples of American creamery butter, found an average water content of 13.9 per cent; salt content, 2.51 per cent; and curd content, 1.18 per cent. This amount of salt in solution in the water present forms, therefore, approximately a 13 per cent brine, which represents the brine formed by adding 18 parts of salt to 100 parts of water. If the same water content be assumed and the salt content found be 1 per cent, the brine present is 5.1 per cent (made by adding 7.1 parts of salt to 100 parts of water); with a salt content of 2 per cent, this strength would be 10.2 per cent; with 3, 15.3 per cent; and with 4, 20.4 per cent. For purposes of mold growth the strength of the brine found is one very significant factor. Another factor is represented by the distribution of air and moisture throughout the mass of butter, and still a third by the relative humidities to which the butter is subjected.

To obtain more complete cultural data for comparison, a series of cultures was made with media containing known percentages of salt. For this purpose 6.5 per cent of salt was introduced into one lot of Czapek's agar (Dox, 1910) and 14 per cent in a second lot. The first represents approximately the proportion of brine in butter with 1.3 per cent of salt, the second the brine with a content of about 2.8 per cent of salt. These cultures were grown in a moist chamber to eliminate the concentration of the brine by drying. The cultural results are given in Table IV.

TABLE IV.—*Cultural results with media containing known percentages of salt*[a]

Mold.	Percentage of salt.		Mold.	Percentage of salt.	
	6.5	14.4		6.5	14.4
Alternaria sp. 3515.....	0.7	0.2	Penicillium roqueforti 3515C	1.0	[b] 1.0
Alternaria sp. 3513.....	.7	.2	Penicillium expansum.	.9	.4
Alternaria sp. 3546.....	.7	.2	Penicillium stolonifera, var	1.0	[b] .7
Cunninghamiella sp...	.6	0	Penicillium chrysogenum.....	.9	.7
Fusarium sp.....	.4	0	Penicillium purpurogenum.....	.....	0
Mucor sp. 3513.....	.7	0	Red mold 3536.3.....	.3	0
Mucor sp. 3514D4.....	.6	0	Rhizopus nigricans....	.7	0
Mucor sp. 3514C1	.6	0	Trichoderma sp.....	.3	0
Mucor sp. 3532.....	.5	0			
Oidium lactis.....	.1	0			
Penicillium sp. 3529a..	1.0	[b] 1.0			
Penicillium roqueforti.	.9	[b] .4			

[a] A typical colony is designated as 1.0; lesser growth by decimal fractions.
[b] These cultures developed slowly, but finally reached the condition indicated.

These cultural results agree with other data published recently (Thom, 1914). Two more series of cultures were made, containing approximately 18 and 21 per cent of salt. In these such organisms as produced marked growth with a salt content of 14.4 per cent were carried, together with other species of Penicillium and Aspergillus. *Penicillium chrysogenum*, *P. stoloniferum*, *Penicillium* sp. 3529a and *Aspergillus repens* produced considerable growth with 18 per cent of salt. Three other organisms produced slight growth. With 21 per cent of salt no colonies were obtained, although spores of *P. chrysogenum* germinated. Comparison with the results of butter inoculation shows that Czapek's solution sustained much larger growth than butter containing comparable percentages of salt. To show the results of these culture series for the organisms obtained from butter, the graphic representation (fig. 1) was prepared. The four series reported were calculated as representing approximately brine conditions in butter containing 1.3, 2.7, 3.4, and 4.1 per cent of salt. Even under the very favorable conditions offered by the culture media, temperature, and humidity used, the mold growth found in the second series was small and in the third series was negligible.

DISCUSSION OF RESULTS

From the data already given, mold is seen to attack the butter itself if unsalted or very lightly salted. Normally salted butter may be affected by green mold only if held under conditions very favorable to mold growth. In general such losses are not great. Both the species of Oidium with its orange-yellow patches and the smudges of Alternaria disappear promptly when even very moderate salting is practiced. These are the important factors in losses of unsalted butter as studied by Jensen (1901 and 1908). Since *Oidium* sp. penetrates the mass of butter

and produces marked discoloration, as well as bad flavors, salting, if practiced at all, should be heavy enough to eliminate this group of organisms. Green molds may damage normally salted butter if cracks and open spaces are left by bad packing. In most cases such mold will be confined to liners and containers if the packing is fairly well done. Rogers (1906) found that paraffining the tubs or boxes used prevented mold on both container and liner. The paraffin prevented the escape of water which would leave the air spaces necessary for mold growth, thus

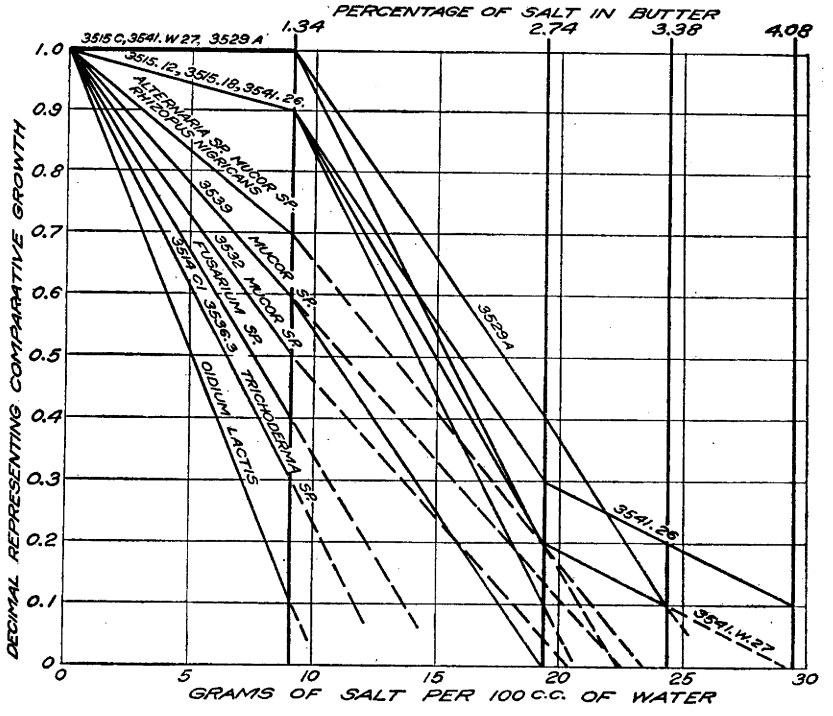

FIG. 1.—Graph showing the effect of salt on molding. Cultural results with organisms obtained from butter. Nos. 3515C, 3541W27, 3929a, 3515.12, 3515.18, and 3541.26 were species of Penicillium. No. 3514C1 was a species of Mucor and No. 3536.3 was the sterile red mold from butter, The dotted portions of the graph represent hypothetical courses for organisms disappearing at percentages not determined but limited by the next experiment.

preventing also loss of weight from the butter itself. Previous papers have taken no account of the presence of mold spores in the butter itself. All possible treatment of containers will fail unless conditions are produced which will prevent the growth of these spores. The same conditions which stop the growth of molds present on the paper and wood of the package also prevent the spores in the butter from growing.

In all storage of butter the temperature factor must not be neglected. Mold growth is progressively reduced by low temperatures. Work elsewhere reported shows that species of Penicillium (Thom, 1910, p. 92, 93, 105) grow very slowly as the temperature approaches the freezing point.

If, as in butter, the fluid present is a strong brine, the temperature must be actually carried considerably below the freezing point of water to eliminate danger from the growth of micro-organisms. Temperatures a few degrees above freezing accompanied, as they frequently are, by moist conditions are favorable to molding in butter. Unsalted butter is more subject to deterioration from micro-organisms than salted butter. Successful storage of such butter is therefore even more dependent upon scrupulously clean dry refrigeration at low temperatures than is the case with salted butter. Cellars and ice refrigeration rarely furnish conditions which will prevent mold growth in unsalted or low-salted butter, although such growth may be delayed or reduced. Butter properly made and salted normally, as indicated above, will not show mold under reasonably careful handling.

SUMMARY

(1) Mold in butter usually takes three forms:

a. Orange-yellow areas with a submerged growth of mycelium are produced by *Oidium lactis.*

b. Smudged or dirty-green areas either entirely submerged or with some surface growth are produced by species of Alternaria and Cladosporium.

c. Green surface colonies are produced by species of Penicillium, or, more rarely, Aspergillus, either upon the butter, causing decomposition, or upon the container or wrappings, injuring the appearance of the sample in the market.

(2) Species of Oidium, Alternaria, and Cladosporium can not develop in butter containing 2.5 per cent of salt. The occurrence of any of these forms in a sample of butter indicates low salting.

(3) Excess of curd favors mold growth. Well-washed butter is less subject to mold.

(4) Leaky butter—butter from which water of buttermilk exudes and collects in the wrappings or in the container—furnishes the best conditions for the beginning of mold growth. From these wet areas colonies may spread to the butter itself.

(5) Wet surfaces, wet wrappings, or high humidity are essential to mold growth in butter. Mold will not grow upon the surface of a piece of butter exposed to humidities of 70 per cent or lower. The water in the butter is thus not sufficiently available to the mold to support the development of a colony, unless evaporation is reduced by high humidities. In closed packages, wet or damp cellars, or carelessly packed masses with cracks or fissures in which moisture collects, mold may seriously injure the appearance of butter packages or actually induce great changes in the butter itself.

(6) Salt up to 2.5 to 3 per cent in butter is sufficient to eliminate mold or reduce it to negligible amount. This is equivalent to the use of a 12 to 15 per cent brine.

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